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Interaction of Proteins with Grapefruit Furanocoumarins

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Grapefruit or grapefruit juice (GFJ) interferes with the cytochrome P450 3A4 activity responsible for metabolizing certain medications. This interference is referred to as the "grapefruit–drug interaction." Previously, we observed that a number of foods sequester many of the furanocoumarins (FCs) in GFJ. In this study, the interactions between macromolecules and GFJ FCs were investigated to determine which food components are responsible for the previously found sequestration. When we removed lipids from corn and salmon by extractions with organic solvents, the resulting defatted corn and salmon powders still sequestered the FCs in GFJ. Further binding experiments with cellulose and pectin showed that these carbohydrates did not interact with GFJ FCs. In contrast, isolated proteins from salmon differentially interacted with GFJ FCs, in which tighter interactions with bergamottin occurred, compared to 6′,7′-dihydroxybergamottin. Overall, our results suggest that the previously observed sequestration of GFJ FCs by foods is likely due to protein–FC interactions.

Grapefruit (*Citrus paradisi* Macf.) is widely consumed and has a number of purported beneficial effects against chronic human diseases (Kiani and Imam, 2007). However, despite these protective effects, the grapefruit–drug interactions first reported by Bailey et al. (1991) have negatively affected the consumption of grapefruit in the U.S. (Mertens-Talcott et al., 2006). In these interactions, grapefruit juice furanocoumarins (GFJ FCs) irreversibly inhibit the human intestinal cytochrome P450 3A4 isozyme (CYP3A4) involved in the metabolism of certain widely-prescribed medications (Guo et al. 2000).

Recently, we attempted to develop methods to remove FCs from GFJ in order to reduce the levels of "grapefruit–drug interactions" occurring in humans after GFJ consumption. Our first attempt involved developing an efficient removal of FCs from GFJ using heat-inactivated fungal hyphae. (Myung et al., 2008a, 2008b). Subsequently, this removal of FCs from GFJ was observed using a variety of other dried foods (Myung et al., unpublished results). To better characterize this removal phenomenon, we conducted preliminary characterizations of the interactions between GFJ FCs and the different macromolecular components of biological tissues, including mainly, lipids, carbohydrates and proteins.

Materials and Methods

FOOD AND GFJ PREPARATION. Fresh salmon and frozen precooked corn were purchased from a local grocery store. Salmon was sliced, and cooked in a microwave for 3 min. The grease and water were drained. The cooked salmon and frozen corn were dried under vacuum at 40 °C for 24 h. The dried samples were ground to powders which were used as starting materials. The GFJ sample was prepared by mixing commercial grapefruit juice concentrate (355 mL) with 1.5 L water, followed by a pH adjustment to 5.0 with KOH to minimize hydrolysis of FCs in GFJ.

INVESTIGATION OF LIPID–FC INTERACTION. Lipids were removed from the corn and salmon powder samples according to Folch et al. (1957). Briefly, the samples were extracted three times with 200 mL chloroform:methanol (2:1) and twice with 100 mL hexane:isopropanol (3:2). Lipids from the samples were completely removed as evidenced by the absence of lipids in the final extracts applied to TLC plates developed with chloroform : methanol : water (60 : 30 : 5) for polar lipids and hexane:diethyl ether:acetic acid (80:20:1.5) for nonpolar lipids (Touchstone, 1995). Fifty mL of GFJ was mixed with 2 g of defatted samples and shaken at 25 °C for 8 h at 300 rpm to ensure maximum binding. The mixtures were subsequently vacuum-filtered through a Whatman No.1 filter. The FCs in the resulting GFJ filtrates or bound to defatted samples were extracted and analyzed by HPLC, as previously described (Myung et al. 2008a).

INVESTIGATION OF CARBOHYDRATE–FC INTERACTION. The major plant carbohydrates, cellulose and pectin were commercially purchased (Sigma, St. Louis, MO). Fifty milliliters of GFJ was mixed with 2 g of cellulose or pectin for 4 h, and filtered through Whatman No.1 filters. The resulting carbohydrate samples and filtrates were subjected to HPLC analysis as described above.

INVESTIGATION OF PROTEIN–FC INTERACTION. Five grams of defatted salmon powder was homogenized with 50 mL of 25 mM potassium phosphate buffer (pH 7.25), and the homogenate was stirred for 2 h at 4 °C. The resulting slurry was centrifuged at 10,000 g for 15 min, and the resulting supernatant was decanted and saved as a crude protein extract. The crude protein extract was collected three times by re-homogenizing, stirring, centrifuging the remaining pellet residue with same volume of the phosphate buffer. These supernatants were precipitated with ammonium sulfate (80% saturation) and the precipitate was dissolved in 20 mL 25 mM potassium phosphate buffer and dialyzed against 1 L of the same buffer. The dialyzed protein solution in 15 mL phosphate buffer (10 mg·mL^{-1}) was added to 15 mL GFJ

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concentrate, and the resulting GFJ/salmon protein sample was shaken for 18 hr at 4 °C. The control, untreated GFJ was mixed with same volume of phosphate buffer and subjected to the same preparation procedures. Control and salmon protein-treated GFJ samples were centrifuged at 10,000 g for 20 min, and the resulting supernatant was further clarified through 0.45 μm PTFE membrane filters (SUN-SRi, Rockwood, TN). Two milliliter filtered GFJ samples were applied to a gel filtration column (2.5 × 33 cm) prepared with Sephadex® G-100 (Sigma). Fractions (1.2 mL per fraction) were collected after the void volume (25 mL) indicated by blue dextran 2000 (Pharmacia, Uppsala, Sweden) at a flow rate of 0.3 mL·min^{-1}, and proteins in these fractions were analyzed by Bradford protein assay (Bradford, 1976). Fractions containing proteins were combined as a pooled protein fraction, while fractions not containing proteins were combined as a non-protein fraction. The FC content of each of these fractions was analyzed by HPLC.

Statistical procedures. All experiments in this study were conducted with three replicates. Analysis of variance and Fisher's least significant difference (LSD) at $P = 0.05$ were applied to determine significant differences among the means of controls and treatments using STATISTICA 7.0 (Statsoft Co., Tulsa, OK).

Results and Discussion

The binding of two GFJ FCs (bergamottin (BM) and 6′,7′-dihydroxybergamottin (DHB)) to food lipids was examined by measuring the sequestration of these compounds by whole food samples vs. defatted samples. The binding of the DHB in 50 mL portions of GFJ to 2 g defatted corn was approximately 25% higher compared to non-defatted corn ($P > 0.05$) while 15% less DHB was bound to defatted salmon compared to untreated salmon ($P > 0.05$) (Fig. 1). In a similar manner, only slight differences ($P > 0.05$) were observed for the binding of BM to defatted corn and salmon powders compared to the original non-defatted food materials (Fig. 1). These results indicate that major portions of binding of GFJ FCs to foods did not occur with lipids.

The efficient binding of the FCs in GFJ by dried salmon powder (Myung et al., unpublished results), lacking structural carbohydrates, also provides an indication that carbohydrates have little role in the FC binding by foods. To verify this, commercially obtained cellulose and pectin were tested for their abilities to sequester FCs from GFJ. As expected, these powders did not adsorb the FCs in GFJ (Table 1). Therefore, it can be concluded that major portions of GFJ FCs binding to foods do not occur with the main lipid and carbohydrate fractions.

Table 1. Concentrations of 6′,7′-dihydroxybergamottin (DHB) and bergamottin (BM) in 50 mL grapefruit juice (GFJ) after mixing with 2 g of cellulose, pectin, or cell wall isolated from *Agaricus bisporus*.

	Concentrations of compounds (μg/mL GFJ)	
	DHB	BM
Non-treated	2.97 NS[z]	0.32 NS
Cellulose	2.60 NS	0.30 NS
Pectin	2.44 NS	0.28 NS

[z]NS denotes a nonsignificant difference between means of triplicates within columns by least square difference (LSD, $P < 0.05$).

The soluble dietary protein fraction recovered from the defatted salmon was added to GFJ, and the potential FC–salmon protein interactions were investigated using gel filtration chromatography (Fig. 2). When run separately, the soluble salmon proteins were

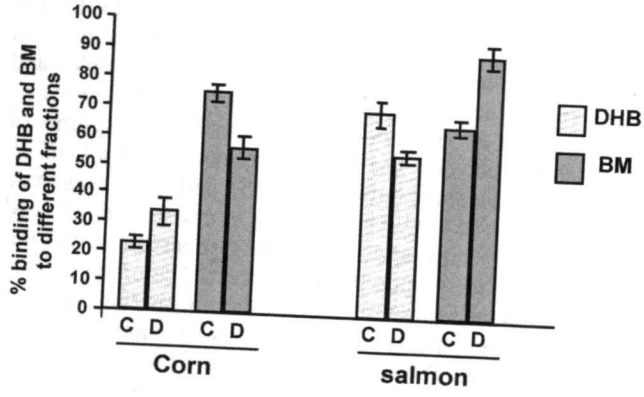

Fig. 1. Binding of DHB and BM to control (nondefatted) and defatted corn and salmon dried powder. Bars represent standard errors of means. C = control; D = defatted.

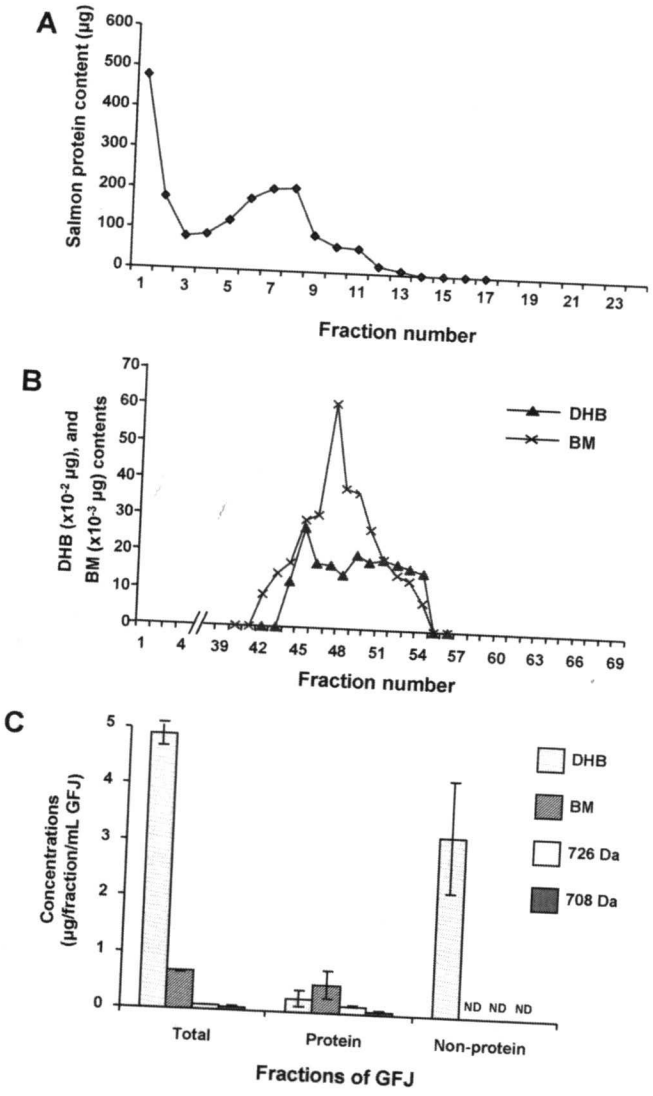

Fig. 2. Fractionations of total salmon proteins after gel filtration chromatography (**A**) fractionations of DHB and BM in GFJ after gel filtration chromatography (**B**), and contents of DHB, BM, and 726 and 708 Da dimers in protein or non-protein fractions after gel filtration of salmon protein-treated GFJ (**C**). Bars represent standard errors of means. ND = not detected.

detected in earlier eluting fractions compared to the complete profile of FCs in GFJ in later-eluting fractions (Fig. 2 A and B). These results validated that proteins and FCs were separable by the Sephadex® G-100 gel filtration column used in these experiments. When the filtered GFJ–salmon protein mixture was run on the same column, only approximately 7% of the DHB eluted with the main salmon protein fractions while most of the DHB eluted in the non-protein fractions (Fig. 2C). In contrast, the more lipophilic BM, and other structurally-related 726 and 708 Da dimers of BM, co-eluted in the main salmon protein fraction, and were absent in the later-eluting non-protein fractions (Fig. 2C). This efficient binding of these lipophilic FCs to proteins, particularly soluble proteins, suggests an importance in polarity of the GFJ FCs in food protein binding.

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